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# Evaluation of Implantable Glucose Enzyme-Based Sensors with Extracorporal Blood Shunt

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**Summary**

This paper presents several methods and instruments developed for the characterization of enzymatic membranes and enzyme electrodes:

1. amplitude and response time of steady-state and transient responses to glucose, automatically determined with an Apple II based workstation,

2. enzyme electrode response to *in vitro* simulated I.V.G.T.T. and to *in vivo* I.V.G.T.T. when connected to an extracorporal blood shunt of conscious rats.

**Key-Words:** *Biosensor, Glucose Electrode, GOD Membrane, Blood Shunt, I.V.G.T.T.*

## Introduction

Among specific electrodes, enzyme-based sensors are probably the most specific and versatile but their operating behaviour is very complex (Thévenot 1985). Their ability to monitor metabolites and especially glucose concentration has been demonstrated since the pioneer work of Clark and Lyons (1962). The major interest and possibilities of such biosensors for *in vivo* implants (Turner and Pickup 1985; Velho, Reach and Thévenot 1987) have led several groups to spent time and efforts for a better understanding of their operating properties. This paper presents briefly our recent strategies for *in vitro* and *in vivo* evaluation of glucose sensors using glucose oxidase (GOD) membranes and anodic detection of enzymatically generated hydrogen peroxide.

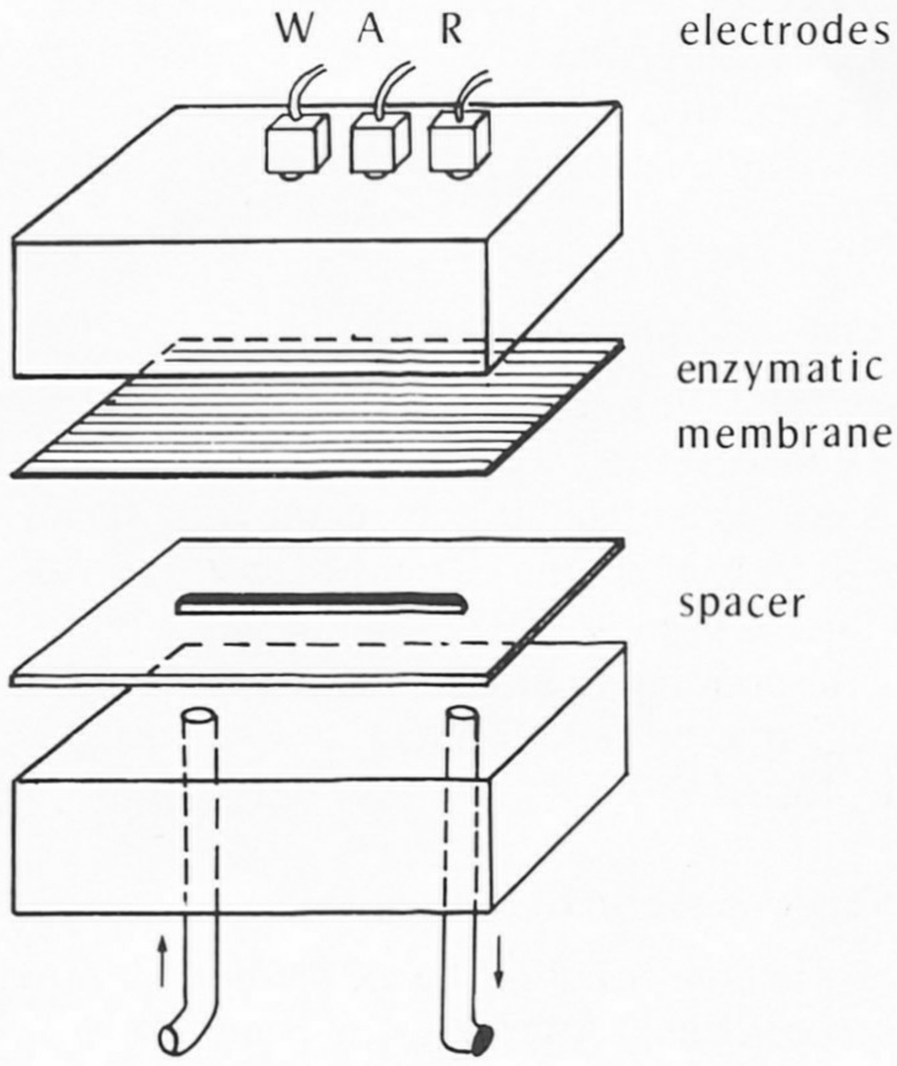


Fig. 1 Schematic of electrochemical glucose sensor using modified LC-EC detector.

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Table 1 Main analytical parameters of 3 different types of GOD membranes and related glucose sensors used in extracorporal blood shunts.

| Parameters | Membrane Type 1 | Type 2 | Type 3 |
|---|---|---|---|
| Membrane material | reconstituted collagen | collagen + cellulose acetate | cellulose acetate |
| Immobilization procedure | acyl azide activation | acyl azide activation | entrapment |
| Membrane thickness (micro. m) | dry: 100<br>swollen: 200–400 | 100 + 15<br>300 + 15 | 5–25<br>5–25 |
| Glucose responses: | | | |
| – sensitivity+ (micro. A.M.-1.mm-2) | 90 | 0.7 | 0.5–2.0 |
| – linear range (mM) | | | |
| lower range | 0.0001 | 0.1 | 0.1 |
| upper range | 2–3 | 40 | 10–30 |
| – response time | | | |
| transient (sec) | 20–50 | 50–90 | 10–60 |
| steady-state (min) | 2–4 | 3–6 | 0.5–3 |
| – stability (days)* | 120–2000 | | 4–8 |

+all sensor responses are referred to anode area.
*stabilities are evaluated for a 50% decrease of sensitivity, either (lower value) under operation or (higher values) in storage conditions.

## Material and Methods

### 1. Enzyme electrodes

All enzyme electrodes used in this study consist of glucose oxidase (GOD) membranes maintained in close contact with a platinum disk of a modified LC-EC detector (Solea Tacussel type DEL-1) (Fig. 1). Enzymatic membranes are either prepared by acyl-azide activation of reconstituted collagen films (Thévenot, Sternberg, Coulet, Laurent and Gautheron 1979) or by entrapment of enzyme in cellulose acetate films (Sternberg, Tallagrand and Thévenot 1983). Solution is circulated to the cell using a Gilson Miniplus II peristaltic pump at a flow rate ranging between 0.1 and 2 ml/min.

### 2. Analytical evaluation of sensors

All assays were performed by step increase or decrease of glucose concentration within the 2.5–20 mM range. Responses are calculated either by comparing steady-state current to background current $I_{bg}$ prior to any glucose addition or by substracting the steady-state current corresponding to previous addition: thus either $I - I_{bg}$ *vs* C or delta.I/delta.C *vs* Cor log C curves are plotted.

### 3. Biological evaluation of sensor

The sensor was connected to the extracorporal blood loop of a conscious rat using a jugular vein and a carotid artery. Total volume of the shunt was about 1.5 ml. Sensor output current was recorded during successive hyperglycemic tests stimulated by venous injection (0.5 g/kg) and blood glucose levels were simultaneously determined using a Beckman Glucose Analyser.

### Analytical Evaluation of the Glucose Sensor

A modified LC-EC cell was used to evaluate GOD membrane properties: the membrane was placed in the cell between the sensing electrodes and the teflon spacer (Fig. 1). Enzymatically generated hydrogen peroxide was immediately detected by anodic amperometry on platinum disk. For routine operations an Apple II+ or IIe based workstation was used both to control glucose standard addition with an electronic buret, to record sensor output signal and to determine automatically steady-state and transient responses and response times (Thévenot, Tallagrand and Sternberg 1987).

GOD membranes were submitted to a simulated I.V.G.T.T. by varying input glucose concentration levels between 2.5 and 20 mM and then, by steps, between 20 and 5 mM. Such experiences lasted for several hours and sensor stability, reproductibility and response times were estimated in conditions similar to extracorporal blood shunt assays. As GOD collagen membranes presented too low values of linear range (Table 1), they were covered with a thin non-enzymatic CA membrane: these superposed membranes presented usually too large response times and gave poor mechanical stability at 37°C. Thus entrapped-GOD CA membranes were preferred for *in vivo* assays.

## Biological Evaluation of the Glucose Sensor

Prior to any *in vivo* assays of flow-through glucose sensors, GOD membranes were tested at 37°C and pH 7.4 and selected for:

- a linear range of calibration curves reaching at least 15 mM,
- a steady state response time lower than 3–4 min,
- a sensitivity reaching 1 microA. $M^{-1} \cdot mm^{-2}$,
- a good membrane semipermeability, yielding responses at least 10 times larger for small neutral molecules, such as hydrogen peroxide, than for small anions, such as ascorbate.

Using GOD membranes which gave acceptable parameters with these flow-through sensors, we performed *in vivo* I.V.G.T.T. on conscious rats. The response characteristics of the glucose sensor was first evaluated *in vitro*, i.e. in circulating buffer where the levels of glucose are augmented in steps. Then the sensor was connected to a rat through catheters placed into a jugular vein and a carotid artery. The concentration of glucose in the blood was changed by intravenous injection of glucose into the catheter. After such *in vivo* measurements, the loop was rinsed and a second *in vitro* calibration was made. As frequent blood samples were determined with a Beckman Glucose Analyser, it was possible to correlate sensor output current to blood glycemia (Fig. 2).

Entrapped-GOD CA membranes demonstrated a good *in vivo* linearity if the highest glucose values, i.e. just after glucose injection, were skipped:

$$I\ (nA) = 2.22 + 0.331\ (Glucose, mM) \quad r^2 = 0.954$$

(Fig. 2).

Using an *in vivo* calibration with initial blood glycemia, glucose concentrations determined by sensor were well correlated with Beckman reference values. These experiments show that the sensor response time is too large to follow the very rapid blood glucose increase after venous injection but that their operating behaviour is satisfactory. Furthermore, as post-calibration curves were significantly lower than pre-calibration ones, their slope decreasing of 50 to 80% for glucose and 30 to 75% for hydrogen peroxide responses respectively, a partial membrane and/or anode inactivation is probably occurring when undiluted blood is in direct contact with the sensor's enzyme membrane. Similar but more limited sensor inactivation (5–20%) had been found previously with GOD-collagen membranes and diluted whole blood: such inhibition was totally reversible, initial sensor sensitivity being reached after rinsing with buffer solution (Sternberg, Apoteker and Thévenot 1980).

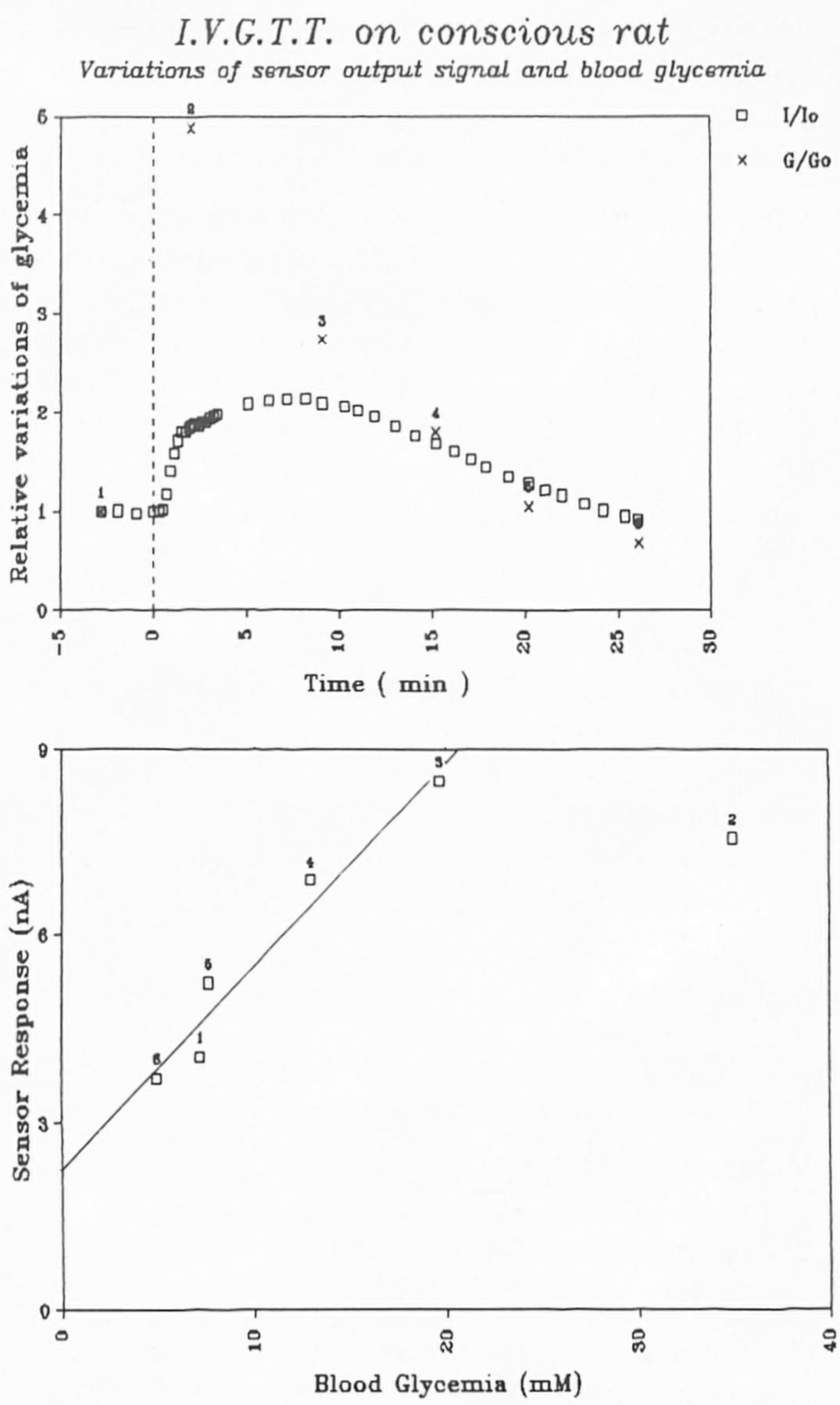


Fig. 2 Biological evaluation of the glucose sensor with extracorporal blood shunt on a conscious free moving rat, during I.V.G.T.T.: (upper curves) relative variations of sensor output current and blood glycemia, and (lower curve) correlation of in vivo glucose sensor current and reference values, using a Beckman Glucometer.

## Conclusion

This presentation of evaluation procedures for glucose sensors based on GOD membranes show the importance of a systematic determination of several analytical parameters and demonstrates the interest of a versatile cell assembly and workstation to perform such routine assays.

Entrapped-GOD CA membranes may be easily prepared by film casting, but their enzyme activity and stability is probably too limited for the development of implanted glucose sensor (Table 1): higher surface activity and more stable covalent GOD coupling seem necessary. An outer membrane or layer seems also necessary both to limit any GOD leakage into blood or body fluid, and to restrict diffusion of glucose thus increasing the upper limit of the sensor linear range (Mullen, Keedy, Churchouse and Vadgama 1986). Although they are difficult to calibrate directly and may give some physiologically inherent time lag, subcutaneous sensors seem preferable to venous ones;

since the original work of Shichiri and Kawamori (1983) several research groups have worked on needle-type microsensors and tested several fabrication procedures (Müller, Abel and Fischer 1986).

Thus a change of strategy has been introduced within our research group. New multilayer membranes are developped using GOD either covalently coupled or copolymerized on CA membranes: they present much higher GOD activities and stabilities (Velho, Reach and Thévenot 1986; Thévenot, Sternberg, Bindra and Wilson 1987) and they can be adapted to the fabrication of needle-type glucose microsensors.

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